Natural Occurrence of Fumonisins in Rice with Fusarium Sheath Rot Disease

H. K. Abbas, USDA-ARS, SWSL, Stoneville, MS 38776; **R. D. Cartwright,** CES, University of Arkansas, Little Rock 72203; **W. T. Shier,** College of Pharmacy, University of Minnesota, Minneapolis 55455; **M. M. Abouzied** and **C. B. Bird,** Neogen Corp., Lansing, MI 48912; **L. G. Rice** and **P. Frank Ross,** USDA-APHIS, NVSL, Ames, IA 50010; **G. L. Sciumbato,** Delta Research and Extension Centers, Stoneville, MS 38776; and **F. I. Meredith,** USDA-ARS, RB Russell Agricultural Research Center, Athens, GA 30604

ABSTRACT

Abbas, H. K., Cartwright, R. D., Shier, W. T., Abouzied, M. M., Bird, C. B., Rice, L. G., Ross, P. F., Sciumbato, G. L., and Meredith, F. I. 1998. Natural occurrence of fumonisins in rice with Fusarium sheath rot disease. Plant Dis. 82:22-25.

Twenty samples of rough rice (*Oryza sativa*) (unpolished kernels) collected during the 1995 harvest season from Arkansas (seven samples) and Texas (13 samples) were obtained from rice fields known to include plants with symptoms of Fusarium sheath rot putatively caused by *Fusarium proliferatum.* Samples were analyzed for fumonisin B_1 (FB_1) at three laboratories using three different extracting solvents by high-performance liquid chromatography (HPLC) or enzyme-linked immunosorbent assay (ELISA) methods. Forty percent of the samples were positive for FB_1 at levels ≤4.3 µg/g by HPLC. The same samples contained FB_1 at ≤3.6 µg/g when measured by an ELISA method. Most samples that were positive for FB_1 were positive for fumonisin B_2 (FB_2) and fumonisin B_3 (FB_3) by HPLC at levels ≤1.2 µg/g. Very good agreement was obtained among the two laboratories using HPLC methods and the third using ELISA. Shelling of the unpolished rice results in hull and brown rice fractions. In a sample that contained 4.3 µg/g in whole kernels, the fumonisin level was very high in hulls (≤16.8 µg/g) and low in brown rice (≤0.9 µg/g). Milling of brown rice results in bran and white rice fractions. Fumonisins were found in bran at a level of ≤3.7 µg/g but were below the level of detection by HPLC in white rice. The presence of fumonisins (FB_1, FB_2, and FB_3) was confirmed by fast atom bombardment/mass spectrometry. This is the first report of fumonisins in naturally contaminated rice in the United States.

Additional keyword: mycotoxins

Fumonisins are a series of structurally related sphingosine analog toxins (29,37) produced by *Fusarium moniliforme* and other fungi (5,16,18,22,27,30,33). The most abundant and one of the most active members of this series is fumonisin B_1 (FB_1) (1,2,35). Since their discovery in 1988 (16) and the first reports of natural occurrence in corn in 1989 (36) and 1990 (28,32), the fumonisins have rapidly become an important class of mycotoxins. Their natural occurrence in corn has been reported in many geographical areas around the world (9,17,19,24,26,31,32,34). Recently, FB_1 was reported in syrup made from sorghum (14).

The fungi that produce fumonisins are well-known pathogens of a variety of plants (8,11,21,23). During the last 3 years, Fusarium sheath rot has been reported as a disease in the southern United States, where rice is a major crop (11–13). Two cultivars (Bengal and Cypress) of rice are very susceptible to this disease (11,13) caused by *F. proliferatum* (11). *F. proliferatum* produces mycotoxins characteristic of *Fusarium* spp., including fumonisin, moniliformin, and beauvericin (25). Health concerns about FB_1 and other fumonisins in corn has led investigators to quantify their presence in products destined for human or animal use. However, to date, fumonisins have not been reported in rice. We report here detection of fumonisins by high-performance liquid chromatography (HPLC) and competitive direct enzyme-linked immunosorbent assay (CD-ELISA) in rough rice kernels harvested from fields known to include plants with Fusarium sheath rot. We also investigated the levels of fumonisins in various rice fractions produced by shelling and milling.

Corresponding author: H. K. Abbas
E-mail: habbas@ag.gov

Accepted for publication 22 September 1997.

Publication no. D-1997-1118-03R

MATERIALS AND METHODS

Rice samples. Twenty samples of combine-harvested rough rice were collected during the 1995 harvest of commercial rice fields in Arkansas and Texas. Samples of ≥0.5 kg were collected randomly from each field and used for fungal isolation and chemical analysis. Samples were transferred to polyethylene bags and kept at –20°C until analyzed. The number of samples, the cultivar sources, and the collection sites are listed in Table 1.

Disease symptoms. Symptoms of Fusarium sheath rot of rice are as previously described (11,12) and include: (i) blanked or partially blanked panicle with reddish brown to off-white florets or kernels often covered with a white to pinkish white powder consisting of microconidia and conidiophores of *F. proliferatum*; (ii) the flag leaf sheath develops a rapidly enlarging lesion, first dull to dark brown and later off-white to tan with a reddish brown border, that eventually encompasses the entire sheath and may result in death of the leaf blade; (iii) lower leaf sheaths may eventually develop lesions as well, but rarely more than two leaf sheaths show symptoms; and (iv) a dense white to pinkish white powder consisting of microconidia and conidiophores of *F. proliferatum* covers the sheath lesions, especially evident during humid periods.

Fungal isolation. Isolates of *F. proliferatum* were recovered from rice tissue showing characteristic Fusarium sheath rot symptoms using standard methods (3,20). The percentage of symptomatic plants was determined by random grid (quadrant) sampling at 50 locations within each field. A plastic square (625 cm^2) was thrown at random 50 times in each affected field, and symptomatic and nonsymptomatic tillers within the square were counted. The number of tillers with symptoms was divided by the total number of tillers and multiplied by 100 to determine percent incidence. Fields were sampled once. *Fusarium* spp. were identified using published methods (21,23). *F. proliferatum* used in tests were grown from a single spore and maintained on silica gel at 4°C, as described by Windels et al. (38).

Table 1. Rough rice samples, sample numbers, and collection sites used in this study[a]

Cultivar	Sample numbers	Collection site
Bengal	1, 4, 18	Arkansas
Cypress	7, 8, 10, 12	Arkansas
	3, 5, 6, 9, 16, 17, 19, 20	Texas
Gulfmont	13, 15	Texas
Lamont	14	Arkansas
	2, 11	Texas

[a] Sample size ≥500 g each.

Fumonisin analysis. Each sample was ground to the consistency of powder using a grinder (Omni-Mixer, Sorvall, Inc., Newtown, CT) and divided into subsamples of 25 or 50 g each. Three laboratories were involved in the testing of these samples for fumonisins:

Laboratory 1 was the Southern Weed Science Laboratory (SWSL) in collaboration with the University of Minnesota. Samples were analyzed for fumonisins by HPLC according to the method of Shephard et al. (28). Briefly, samples were extracted with methanol:water (1:1, vol/vol), centrifuged, and filtered. The filtrate was applied to a Bond-Elut SAX cartridge and eluted with 0.5% acetic acid in methanol. The solution was evaporated, and the residue was derivatized with *o*-phthaldialdehyde (OPA) and injected onto the HPLC column. The limit of detection was 0.5 µg/g.

Laboratory 2 was USDA-APHIS, NVSL, Ames, Iowa. This laboratory used the method previously described in detail (26). Briefly, samples were extracted with 50% acetonitrile in water (vol/vol) by shaking for 30 min and filtered. The filtrate was purified on C_{18} solid-phase extraction columns, and the eluate was derivatized with OPA and injected onto the liquid chromatograph column. Limit of detection was 0.5 µg/g.

Laboratory 3 was Neogen Corporation. Samples were extracted with 70% (vol/vol) methanol:water and filtered. CD-ELISA was used to determine total fumonisins. This procedure has been described in detail by Abouzied et al. (6) and has a limit of detection of 0.1 µg/g.

Identity confirmation. The identity of toxins present in harvested rice samples was confirmed by fast atom bombardment/mass spectrometry (FAB/MS) (5) or continuous flow/fast atom bombardment/mass spectrometry (CF/FAB/MS) (18) at the University of Minnesota, St. Paul. High resolution fast atom bombardment/mass spectrometry (HR FAB/MS) was performed to confirm the elemental formula of each toxin in rice samples 3, 6, 16, and 17, at the U.S. Food and Drug Administration, Washington, DC.

Distribution of FB_1 in shelling and milling fractions. One kg of the unpolished rice sample 3-2 was used for this study. Shelling and milling of unpolished rice was performed at the quality control laboratory at Uncle Ben's Rice Company (Greenville, MS) using the standard commercial procedure. In the shelling process, the rice sample was fractionated into brown rice and hulls. In the milling process, bran was separated from brown rice, leaving the white rice fraction.

RESULTS

Fumonisin B_1 was found in readily detectable levels (>1 µg/g) in six out of 20 (30%) independent rice samples (Table 2). As determined by HPLC at SWSL and the University of Minnesota (lab 1), the six positive samples had levels of FB_1 ranging from 3.1 to 4.3 µg/g. Including FB_2 and FB_3, total fumonisins ranged from 3.6 to 6.6 µg/g in the same samples. The same six samples independently assayed by HPLC at NVSL in Ames (lab 2) contained FB_1 at 2.3 to 4.0 µg/g, while total fumonisins ranged from 2.3 to 5.4 µg/g.

The presence of total fumonisins in these samples correlated well with percent sheath and panicle symptoms (r = 0.97) and with the percent recovery of *F. proliferatum* from grain obtained from fields infected with Fusarium sheath rot (r = 0.97) (Table 3). Similarly, percent sheath and panicle symptoms correlated well with percent recovery of *F. proliferatum* from grain (r = 0.99).

FB_1, FB_2, and FB_3 were present, as shown by both FAB/MS and CF/FAB/MS. The elemental formula of each was confirmed by FAB/MS. FB_1 levels as determined by CD-ELISA were similar to but slightly lower than HPLC levels. Measurements by the two HPLC methods were highly correlated for FB_1 (r = 0.97), FB_2 (r = 0.94), and FB_3 (r = 0.64). The CD-ELISA measurements were well correlated with the results of HPLC measurements from both laboratory 1 (r = 0.95) and laboratory 2 (r = 0.95). FB_2 and FB_3 could not be measured separately by CD-ELISA because this antibody reacts with all fumonisins to some extent.

One sample (no. 19) was used to determine the fate of fumonisin in the shelling and milling processes. The shelling process divides the unpolished rice into hulls and brown rice, while milling gives fractions of bran and white rice. Shelling substantially reduced FB_1 levels in the brown rice fraction by 75 to 80%, from 4.7, 2.4, and 3.5

Table 2. Fumonisin levels in unpolished rice samples

Sample no.[a]	Laboratory no.[b]	Assay method[c]	Fumonisin level (µg/g)			Total (µg/g)
			FB_1	FB_2	FB_3	
3	1	HPLC	3.5	1.2	0.5	5.2
	2	HPLC	3.0	1.1	ND	4.1
	3	CD-ELISA				3.3
19	1	HPLC	4.3	1.0	0.5	5.8
	2	HPLC	3.3	0.9	0.5	4.7
	3	CD-ELISA				3.1
4	1	HPLC	Trace[d]	ND	ND	Trace
	2	HPLC	0.5	ND	ND	0.5
	3	CD-ELISA				Trace
6	1	HPLC	3.1	0.5	ND	3.6
	2	HPLC	2.3	ND	ND	2.3
	3	CD-ELISA				1.9
20	1	HPLC	3.1	0.8	0.5	4.4
	2	HPLC	2.9	0.7	ND	3.6
	3	CD-ELISA				3.0
9	1	HPLC	0.9	ND	ND	0.9
	2	HPLC	Trace	ND	ND	Trace
	3	CD-ELISA				Trace
16	1	HPLC	4.1	1.0	0.5	5.6
	2	HPLC	4.0	0.9	0.5	5.4
	3	CD-ELISA				3.6
17	1	HPLC	3.3	0.9	0.6	4.8
	2	HPLC	3.1	0.9	0.5	4.5
	3	CD-ELISA				2.9

[a] Fumonisins were not detectable (ND) in the other 12 of 20 samples at 0.5 µg/g when determined by high-performance liquid chromatography (HPLC) or at 1 µg/g when determined by competitive direct enzyme-linked immunosorbent assay (CD-ELISA) (Veratox).
[b] Laboratory 1 included Southern Weed Science Laboratory and the University of Minnesota; laboratory 2 was NVSL, USDA-APHIS; laboratory 3 was Neogen Corp.
[c] CD-ELISA data shown for total fumonisins because of cross-reactivity among fumonisins.
[d] ≤0.3 µg/g.

Table 3. Incidence of Fusarium sheath rot symptom and percent recovery of *Fusarium proliferatum* from designated field and grain samples

Sample	Sheath and panicle symptoms[a] (%)	Recovery from grain[b] (%)
1	1	0
4	5	2
18	<1	0
7	1	1
8	3	5
10	<0.1	1
12	2.5	8
3	20[c]	100
5	…[d]	6
6	20[c]	100
9	3	12
16	20[c]	100
17	20[c]	100
19	20[c]	100
20	20[c]	100

[a] Percentage of symptomatic plants was determined as described in Materials and Methods.
[b] Based on two lots of 100 seeds plated on Nash-Snyder agar (20). Grain was collected from combine samples from each field.
[c] Different areas of same field.
[d] Field survey was not performed.

µg/g in the three laboratories to 0.9, 0.6, and 1.2 µg/g, respectively. Milling gave a white rice fraction with undetectable levels of fumonisins (Table 4). FB_2 and FB_3 were decreased in the shelling and milling processes to undetectable levels in both brown rice and white rice.

DISCUSSION

Fumonisin levels in food and feed crops are an important public health concern. The cause of most human cancer is not known (10), but FB_1 as a putative environmental tumor promoter (16) could contribute to it, especially if exposure is long-term. Fumonisin contamination of animal feed has caused illness and death of livestock, with associated economic losses (27). Guidance levels for fumonisin in animal feed issued by the American Association of Veterinary Laboratory Diagnosticians, Mycotoxin Committee, vary by species, but levels above 5 µg/g can be harmful to horses (15). Rough rice and rice hulls, which are sometimes fed to livestock, contained concentrations of fumonisins above 5 µg/g. White rice, the form of rice most widely consumed in human diets in this country, did not contain detectable levels. Therefore, the fumonisins are localized primarily in the hulls and bran. Nonetheless, this study demonstrates that rice and products derived from rice may potentially contain fumonisins and should be monitored for fumonisins to assure maximum food safety. Fumonisins are heat-stable and would likely not be destroyed by rice cooking methods (4,7). Additional studies are needed to determine the prevalence of fumonisins in rice grown in other geographical areas and in food and feed products derived from rice.

Fusarium sheath rot of rice is a recently reported problem in the United States, and the disease itself remains poorly understood. While reproduction of symptoms under greenhouse conditions has been reported, reproduction of the disease under field conditions using various isolates of *F. proliferatum* has been largely unsuccessful, leading to speculation about conditions needed for infection and disease development. The fungus appears to be a weak pathogen under most conditions, and other factors may be involved in the field. Other conditions result in blanked panicles in U.S. rice fields, and all causes of these symptoms need to be determined and clarified. Nevertheless, the widespread *F. proliferatum* contamination of commercial rice fields growing the newly released cultivars is a cause for concern.

ACKNOWLEDGMENTS

We thank Bobbie J. Johnson, USDA-ARS, SWSL, Stoneville, MS, for her help in preparation this manuscript. We thank C. J. Mirocha and W. Xie for confirming toxins by CF/FAB/MS and T. Krick for confirming toxins by FAB/MS, University of Minnesota, St. Paul; and Mary W. Trucksess and Michael E. Stack, U.S. Food and Drug Administration, Washington, DC., for confirming the presence of fumonisins. We also thank Dorothy B. Davis and David Weddington, Uncle Ben's Rice Company, Greenville, MS, for shelling and milling a rice sample to obtain various fractions. In addition, we thank C. M. Ocamb, USDA Forest Service, St. Paul, MN, and James Correll, Department of Plant Pathology, University of Arkansas, Fayetteville, for confirmation of the identity of *F. proliferatum.*

LITERATURE CITED

1. Abbas, H. K., Boyette, C. D., Hoagland, R. E., and Vesonder, R. F. 1991. Bioherbicidal potential of *Fusarium moniliforme* and its phytotoxin, fumonisin. Weed Sci. 39:673-677.
2. Abbas, H. K., Gelderblom, W. C. A., Cawood, M. E., and Shier, W. T. 1993. Biological activities of fumonisins, mycotoxins from *Fusarium moniliforme*, in jimsonweed (*Datura Stramonium* L.) and mammalian cell cultures. Toxicon 31:345-353.
3. Abbas, H. K., Mirocha, C. J., Kommedahl, T., Vesonder, R. F., and Golinski, P. 1989. Production of trichothecene and non-trichothecene mycotoxins by *Fusarium* species isolated from maize in Minnesota. Mycopathologia 108:55-58.
4. Abbas, H. K., and Riley, R. T. 1996. The presence and phytotoxicity of fumonisins and AAL-toxin in *Alternaria alternata*. Toxicon 34:133-136.
5. Abbas, H. K., Vesonder, R. F., Boyette, C. D., Hoagland, R. F., and Krick, T. 1992. Production of fumonisins by *Fusarium moniliforme* cultures isolated from jimsonweed in Mississippi. J. Phytopathol. 136:199-203.
6. Abouzied, M. M., Askegard, S. D., Bird, C. B., and Miller, B. M. 1995. Development of a rapid quantitative ELISA for determination of the mycotoxin fumonisin in food and feed. J. Clin. Ligand Assay 18:145-149.
7. Alberts, J. F., Gelderblom, W. C. A., Thiel, P. G., Marasas, W. F. O., Van Schalkwyk, D. J., and Behrend, Y. 1990. Effects of temperature and incubation period on production of fumonisin B_1 by *Fusarium moniliforme*. Appl. Environ. Microbiol. 56:1729-1733.
8. Bhargava, S. N., Shukla, D. N., and Singh, N. 1978. *Fusarium moniliforme* causing panicle rot of rice. Ind. Phytopathol. 31:367-369.
9. Bullerman, L. B., and Tsai, W. Y. 1994. Incidence and levels of *Fusarium moniliforme*, *Fusarium proliferatum* and fumonisins in corn and corn-based foods and feeds. J. Food Prod. 57:541-546.
10. Cairns, J. 1981. The origin of human cancer. Nature 289:353-357.
11. Cartwright, R. D., Correll, J. C., and Crippen, D. L. 1995. Fusarium sheath rot of rice in Arkansas. (Abstr.) Phytopathology 85:1199.
12. Cartwright, R. D., Lee, F. N., Crippen, D. L., and Templeton, G. E. 1993. Monitoring of rice diseases under different locations and cultural practices in Arkansas. Pages 86-100 in: Arkansas Rice Research Series. D. R. Wells, ed. Ark. Agric. Exp. Stn. Res. Ser. 439. University of Arkansas, Fayetteville.
13. Chen, M. J. 1957. Studies on sheath rot of rice plant. J. Agric. Taiwan 6:84-102.
14. Cho, T. H., Trucksess, M. W., and Page, W. S. 1996. High performance liquid chromatographic determination of fumonisin B_1 in sorghum syrup. (Abstr.) Page 58 in: AOAC Intl. Annu. Meeting Exposition, 110th.
15. Diener, U. L. 1996. Mycotoxicology Newsl. 7(1):3.
16. Gelderblom, W. C. A., Jaskiewicz, K., Marasas, W. F. O., Thiel, P. G., Horak, R. M., Vleggaar, R., and Kreik, N. P. J. 1988. Fumonisins - novel mycotoxins with cancer-promoting activity produced by *Fusarium moniliforme*. Appl. Environ. Microbiol. 54:1806-1811.
17. Hopmans, E. C., and Murphy, P. A. 1993. Detection of fumonisins B_1, B_2, and B_3 and hydrolyzed fumonisin B_1 in corn-containing foods. J. Agric. Food Chem. 41:1655-1658.
18. Mirocha, E. J., Chen, J., Xie, W., Abbas, H. K., and Hogge, L. H. 1996. Biosynthesis of fumonisin and AAL derivatives by *Alternaria* and *Fusarium* in laboratory culture. Pages 213-224 in: Fumonisin in Food. L. S. Jackson, J. W. DeVries, and L. B. Bullerman, eds. Plenum Press, New York.
19. Murphy, P. A., Rice, L. G., and Ross, P. F. 1993. Fumonisins B_1, B_2, and B_3 content of Iowa, Wisconsin and Illinois corn and screenings. J. Agric. Food Chem. 41:263-266.
20. Nash, S. M., and Snyder, W. C. 1965. Quantitative and qualitative comparisons of *Fusarium* populations in cultivated fields and noncultivated parent soils. Can. J. Bot. 43:939-945.
21. Nelson, P. E. 1992. Taxonomy and biology of

Table 4. Fumonisin levels in rice fractions from field sample no. 19

Rice fraction	Laboratory no.[a]	Assay method	Fumonisin levels (µg/g)[b]			Total (µg/g)
			FB_1	FB_2	FB_3	
Unpolished rice	1	HPLC	4.7	1.0	0.6	6.3
	2	HPLC	2.4	0.7	1.3	4.4
	3	CD-ELISA[c]				3.5
Hulls	1	HPLC	13.0	2.6	1.2	16.8
	2	HPLC	8.4	1.3	1.6	11.3
	3	CD-ELISA				14.5
Brown rice	1	HPLC	0.9	ND	ND	0.9
	2	HPLC	0.6	ND	ND	0.6
	3	CD-ELISA				1.2
Bran	1	HPLC	3.1	0.6	ND	3.7
	2	HPLC	1.7	0.5	0.5	2.7
	3	CD-ELISA				3.4
White rice	1	HPLC	ND	ND	ND	ND
	2	HPLC	ND	ND	ND	ND
	3	CD-ELISA				ND

[a] Laboratory 1 included Southern Weed Science Laboratory and the University of Minnesota; laboratory 2 was NVSL, USDA-APHIS; laboratory 3 was Neogen Corp.

[b] HPLC = high-performance liquid chromatography; CD-ELISA = competitive direct enzyme-linked immunosorbent assay. CD-ELISA data are given for total fumonisins because of cross-reactivity among fumonisins.

[c] ND = no detectable fumonisin at 0.5 µg/g when determined by HPLC or at 1.0 µg/g when determined by CD-ELISA.

Fusarium moniliforme. Mycopathologia 117:29-36.
22. Nelson, P. E., Plattner, R. D., Shackelford, D. D., and Desjardins, A. E. 1992. Fumonisin B_1 production by *Fusarium* species other than *F. moniliforme* in section Liseola and some related species. Appl. Environ. Microbiol. 58:984-989.
23. Nelson, P. E., Toussoun, T. A., and Marasas, W. F. O. 1983. *Fusarium* species: An illustrated manual for identification. Pennsylvania State University, University Park.
24. Pittet, A., Parisod, V., and Schellenberg, M. 1992. Occurrence of fumonisins B_1 and B_2 in corn-based products from the Swiss market. J. Agric. Food Chem. 40:1352-1354.
25. Plattner, R. D., and Nelson, P. E. 1994. Production of beauvericin by a strain of *Fusarium proliferatum* isolated from corn fodder for swine. Appl. Environ. Microbiol. 60:3894-3896.
26. Rice, L. G., Ross, P. F., DeJong, J., Plattner, R. D., and Coats, J. R. 1995. Evaluation of a liquid chromatographic method for the determination of fumonisins in corn, poultry feed, and *Fusarium* culture material. J. AOAC Intl. 8:1002-1009.
27. Ross, P. F., Nelson, P. E., Richard, J. L., Osweiler, G. D., Rice, L. G., Plattner, R. D., and Wilson, T. M. 1990. Production of fumonisins by *Fusarium moniliforme* and *Fusarium proliferatum* isolates associated with equine leukoencephalomalacia and a pulmonary edema syndrome in swine. Appl. Environ. Microbiol. 56:3225-3226.
28. Shephard, G. S., Sydenham, E. W., Thiel, P. F., and Gelderblom, W. C. A. 1990. Quantitative determination of fumonisins B_1 and B_2 by high performance liquid chromatography with fluorescence detection. J. Liquid Chromat. 13:2077-2087.
29. Shier, W. T. 1992. Sphingosine analogs: An emerging new class of toxins that includes the fumonisins. J. Toxicol.-Toxin Rev. 11:241-257.
30. Sydenham, E. W., Rheeder, J. P., Marasas, W. F. O., and Shephard, G. S. 1996. *Fusarium globosum*: A new fumonisin producing species isolated from corn. (Abstr.) Page 59 in: AOAC Intl. Annu. Meeting Exposition, 110th.
31. Sydenham, E. W., Shephard, G. S., and Theil, P. G. 1992. Liquid chromatographic determination of fumonisins B_1, B_2, and B_3 in foods and feeds. J. Assoc. Off. Anal. Chem.75:313-318.
32. Sydenham, E. W., Shephard, G. S., Theil, P. G., Marasas, W. F. O., and Stockenstrom, S. 1991. Fumonisin contamination of commercial corn-based foodstuffs. J. Agric. Food Chem. 39:2014-2018.
33. Theil, P. G., Marasas, W. F. O., Sydenham, E. W., Shephard, G. S., Gelderblom, W. C. A., and Nieuwenhuis, J. J. 1991. Survey of fumonisin production by *Fusarium* species. Appl. Environ. Microbiol. 57:1089-1093.
34. Ueno, Y., Aoyama, S., Sugiura, Y., Wang, D.-S., Lee, U.-S., Hirooka, E. Y., Hara, S., Karki, T., Chen, G., and Yu, S.-Z. 1993. A limited survey of fumonisins in corn and corn-based products in Asian countries. Mycotoxin Res. 9:27-34.
35. Vesonder, R. F., Peterson, R., Plattner, R., and Weisleder, D. 1990. Fumonisin B_1: Isolation from corn culture, and purification by high performance liquid chromatography. Mycotoxin Res. 6:85-88.
36. Voss, K. A., Norred, W. P., Plattner, R. D., and Bacon, C. W. 1989. Hepatotoxicity and renal toxicity in rats of corn samples associated with field cases of equine leukoencephalomacia. Food Chem. Toxicol. 27:89-96.
37. Wang, E., Norred, W. P., Bacon, C. W., Riley, R. T., and Merrill, A. H. 1991. Inhibition of sphingolipid biosynthesis with fumonisins. J. Biol. Chem. 266:14480-14490.
38. Windels, C. E., Burnes, P. M., and Kommedahl, T. 1988. Five-year preservation of *Fusarium* species in silica gel and soil. Phytopathology 78:107-109.